METHODS FOR GENETIC CONTROL OF INSECT INFESTATIONS IN PLANTS AND COMPOSITIONS THEREOF

PRIORITY CLAIM

[0001] This application claims the priority of U.S. Provisional Application Ser. No. 60/718,034, filed Sep. 16, 2005, which application is incorporated herein by reference in its entirety.

[0002] The Sequence Listing is submitted on one compact disc (Copy 1), together with a duplicate thereof (Copy 2), each created on Sep. 15, 2006, and each containing one 669 kb file entitled "MNDE002.APP.TXT." The material contained on the compact disc is specifically incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0003] 1. Field of the Invention

[0004] The present invention relates generally to genetic control of pest infestations. More specifically, the present invention relates to recombinant DNA technologies for post-transcriptionally repressing or inhibiting expression of target coding sequences in the cell of a pest to provide a pest-protective effect.

[0005] 2. Description of Related Art

[0006] The environment in which humans live is replete with pest infestation. Pests including insects, arachnids, crustaceans, fungi, bacteria, viruses, nematodes, flatworms, roundworms, pinworms, hookworms, tapeworms, trypanosomes, schistosomes, botflies, fleas, ticks, mites, and lice and the like are pervasive in the human environment. A multitude of means have been utilized for attempting to control infestations by these pests. Compositions for controlling infestations by microscopic pests such as bacteria, fungi, and viruses have been provided in the form of antibiotic compositions, antiviral compositions, and antifungal compositions. Compositions for controlling infestations by larger pests such as nematodes, flatworm, roundworms, pinworms, heartworms, tapeworms, trypanosomes, schistosomes, and the like have typically been in the form of chemical compositions that can be applied to surfaces on which pests are present or administered to infested animals in the form of pellets, powders, tablets, pastes, or capsules and the like. There is a great need in the art for improvement of these methods and particularly for methods that would benefit the environment relative to the prior techniques.

[0007] Commercial crops are often the targets of insect attack. Substantial progress has been made in the last a few decades towards developing more efficient methods and compositions for controlling insect infestations in plants. Chemical pesticides have been very effective in eradicating pest infestations. However, there are several disadvantages to using chemical pesticidal agents. Chemical pesticidal agents are not selective. Applications of chemical pesticides intended to control invertebrate pests, such as coleopteran insects including corn rootworm species that are harmful to various crops and other plants, exert their effects on non-target fauna as well, often effectively sterilizing a field for a period of time over which the pesticidal agents have been applied. Chemical pesticidal agents persist in the environment and generally are slow to be metabolized, if at all. They accumulate in the food chain, and particularly in the higher predator species. Accumulations of these chemical pesticidal agents results in the development of resistance to the agents and in species higher up the evolutionary ladder, can act as mutagens and/or carcinogens to cause irreversible and deleterious genetic modifications. Thus there has been a particularly long felt need for environmentally friendly methods for controlling or eradicating insect infestation on or in plants, i.e., methods that are selective, environmentally inert, non-persistent, and biodegradable, and that fit well into pest resistance management schemes.

[0008] Compositions that include *Bacillus thuringiensis* (Bt) bacteria have been commercially available and used as environmentally safe and acceptable insecticides for more than thirty years. The insecticidal effect of Bt bacteria do not persist in the environment, are highly selective as to the target species affected, exert their effects only upon ingestion by a target pest, and have been shown to be harmless to plants and other non-targeted organisms, including humans. Transgenic plants containing one or more genes encoding insecticidal Bt protein are also available in the art and are remarkably efficient in controlling insect pest infestation. A substantial result of the use of recombinant plants expressing Bt insecticidal proteins is a marked decrease in the amount of chemical pesticidal agents that are applied to the environment to control pest infestation in crop fields in areas in which such transgenic crops are used. The decrease in application of chemical pesticidal agents has resulted in cleaner soils and cleaner waters running off of the soils into the surrounding streams, rivers, ponds and lakes. In addition to these environmental benefits, there has been a noticeable increase in the numbers of beneficial insects in crop fields in which transgenic insect resistant crops are grown because of the decrease in the use of chemical insecticidal agents.

[0009] Antisense methods and compositions have been reported in the art and are believed to exert their effects through the synthesis of a single-stranded RNA molecule that in theory hybridizes in vivo to a substantially complementary sense strand RNA molecule. Antisense technology has been difficult to employ in many systems for three principle reasons. First, the antisense sequence expressed in the transformed cell is unstable. Second, the instability of the antisense sequence expressed in the transformed cell concomitantly creates difficulty in delivery of the sequence to a host, cell type, or biological system remote from the transgenic cell. Third, the difficulties encountered with instability and delivery of the antisense sequence create difficulties in attempting to provide a dose within the recombinant cell expressing the antisense sequence that can effectively modulate the level of expression of the target sense nucleotide sequence.

[0010] There have been few improvements in technologies for modulating the level of gene expression within a cell, tissue, or organism, and in particular, a lack of developed technologies for delaying, repressing or otherwise reducing the expression of specific genes using recombinant DNA technology. Furthermore, as a consequence of the unpredictability of these approaches, no commercially viable means for modulating the level of expression of a specific gene in a eukaryotic or prokaryotic organism is available.

[0011] Double stranded RNA mediated inhibition of specific genes in various pests has been previously demonstrated. dsRNA mediated approaches to genetic control have been tested in the fruit fly *Drosophila melanogaster* (Kennerdell and Carthew, 1998; Kennerdell and Carthew, 2000). Kennerdell and Carthew (1998) describe a method for delivery of